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# Separation of Phosphatases of Takadiastase by Paper Chromatography

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## ABSTRACTS

### Studies on Barley Malt

#### Some Investigations upon Zymogen Amylase

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(Katagiri Laboratory)

The pH-curve of amylase activity revealed by zymogen amylase which is awoken by papain, is in accord with that of free amylase at the optimum point. But the optimum pH range of zymogen amylase is narrow both on acid and alkaline sides. Thermostability of zymogen amylase is higher than free amylase, since the former maintained almost its activity even when it was treated for 20 min. at 70°C.

We found interesting results in several experiments in which germinated green malt was kept in a closed vessel, containing small amount of toluene as disinfectant, and then left the green malt to autolyse. When this treatment was carried out at the beginning of germination, zymogen amylase was decreased, while free amylase was increased, whereas if it was treated at the later stage of germination, free amylase was decreased and zymogen amylase was remarkably increased. A substance for amylase was frequently found in the autolysed green malt.

Even with non-germinated barley which was lost germinating power by soaking in water at 40°C with toluene, acceleration of saccharifying power and formation of dextrinogen-amylase were observed both in free and zymogen forms, when it was left to autolyse.

( Read at the semi-annual meeting of the Institute on June 11, 1954 )

### Separation of Phosphatases of Takadiastase by Paper Chromatography

Senji UTZINO and Ryokuero SUZUE

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We studied phosphatase action on thiaminetriphosphate by the partition paperchromatography, using filterpaper Toyo Roshi No. 50, (40×40 cm).

The papers were irrigated with 80 % EtOH for ascending chromatography in an all-glass apparatus, usually for 17 hrs. The spots were revealed by Dragendorff's reagent. Rf values were as follows: thiamine 0.50, thiaminemonophosphate (TMP) 0.33, cocarboxylase (TDP) 0.13 and thiaminetriphosphate (TTP) 0.06.

Two lines, apart 5 cm respectively from two adjacent edges of the paper, were drawn and the intersecting point was regarded as the origin. At first a dialysed 50 % Takadiastase solution was spotted at the origin, and developed with 2 % NaCl solution in the refrigerator

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for 6~7 hrs. Then 75 mg. of TTP, 0.75 ml. of AcOH buffer (pH 5.5) and 0.75 ml. of glycerol were applied on the developed line of enzymes. After keeping it in the saturated water vapour at 37°C for 1 hr., they were developed with 80 % EtOH at right angles with the developed line of enzymes. The spots of thiamine appeared in two places, where Rf values were 0 and 0.3~0.4, and that of TMP was 0~0.7. The spots of TTP and TDP were observed on the whole applying line. These results suggest that TTP can be hydrolysed to TDP spontaneously. Organic pyrophosphatase which hydrolyses TDP to TMP develops in the value of Rf 0~0.7, and phosphomonoesterase which hydrolyses TMP to thiamine has the values of Rf 0 and 0.3~0.4. The purified pyrophosphatase (Utzino and Suzue, This Bulletin. 32, 103 (1954) hydrolysed TTP to TMP (Rf=0~0.7) but not to thiamine. The purified urine phosphomonoesterase (Utzino and Suzue, *ibid*, 31, 385 (1953)) did not hydrolyse TTP to TMP and thiamine.

( Read at the semi-annual meeting of the on June 11, 1954 )